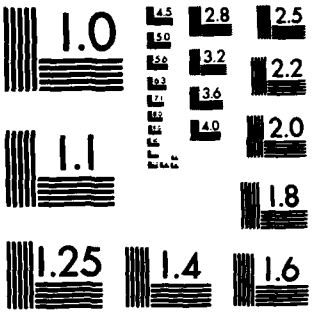
AD-A140 134 GENETICS OF NOVEL HYBRID BACTERIOPHAGE AND DEVELOPMENT 1/1
OF GENERALIZED TRA..(U) HAHNEMANN MEDICAL COLL AND
HOSPITAL PHILADELPHIA PA N YAMAMOTO FEB 80
UNCLASSIFIED DAMD17-79-C-9134 F/G 6/13 NL



AD_____

ANNUAL PROGRESS REPORT

Genetics of Novel Hybrid Bacteriophage and Development of Generalized Transducing System for Salmonella typhosa

Annual Progress Report

Nobuto Yamamoto, Ph.D.

February, 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,

Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9134

Hahnemann Medical College
Philadelphia, Pennsylvania 19102

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD A140134	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* Genetics of Novel Hybrid Bacteriophage and Development of Generalized Transducing System for Salmonella typhosa		5. TYPE OF REPORT & PERIOD COVERED Annual Progress Report (From 9/1/79 to 1/31/80)
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Nobuto Yamamoto		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-79-C-9134
9. PERFORMING ORGANIZATION NAME AND ADDRESS Hahnemann Medical College 230 N. Broad St. Philadelphia, Pa. 19102		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102A 3M161102BS01.00.010
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE February 1980
		13. NUMBER OF PAGES 13
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Bacteriophage, Hybrid bacteriophage, Salmonella typhimurium, Hybrid bacteria, E. coli - S. typhimurium hybrid, Genetic homology, Genome length, Genetic recombination, Antigen conversion, Cell surface, Gene expression, and Bacteriophage tail.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The λimmP22 hybrids, which retain the protein coat of λ, were found to contain various lengths of homology with Salmonella phage P22. The length of the entire λimmP22 genome also varies from strain to strain. Density gradient centrifugation revealed that the genome of a λimmP22dis strain is 5% larger than that of a λimmP22 strain. Hybrids λimmP22dis are heat unstable (at 55 C) whereas λimmP22 are rather stable. Because the λimmP22dis genome is tightly packed in λ porotein coat, λimmP22dis particles are heat unstable. Survivors

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

20. Abstract (Continued)

after heat treatment are often found to be deletion mutants lacking the al and 9 genes of P22.

Hybrids phages between Salmonella Phage P22 and colipahge φ80 have been isolated by using E. coli-S. typhimurium hybrids. Among those hybrid phages, φ80immP22dis hybrids carrying both immunity related genes, immC and immI regions of P22 were isolated. Since P22 tail component gene 9 and somatic antigen conversion gene al are located between the immC and immI regions of P22, we examined whether φ80immP22dis hybrids carry these genes. Some φ80immP22dis hybrids carry gene al but not gene 9 whereas the remaining φ80immP22dis hybrids carry gene 9 only. No φ80immP22 hybrid phages containing both the P22 genes 9 and al were found. These observations suggest that φ80immP22dis hybrid is formed as a consequence of multiple crossovers.

Although λimmP22dis hybrid phages carry both genes 9 and al, φ80immP22dis hybrids carry only one of these genes 9 or al. Since the size of φ80 phage genome is about 92% of the λ genome, we concluded that the φ80immP22 genome is unable to contain both genes 9 and al.

Numerous attempts to isolate hybrids between P22 and coli mutator phage Mμ-1 were unsuccessful. This may be due to lack of induction of Mμ-1 prophage by P22 superinfection although we found that P22 infection of λ or φ80 lysogens results in induction of their prophages. Accordingly we prepared lysogens with temperature inducible (cts) mutants of Mμ-1 phage. When P22 phage stocks were prepared by P22 infection of temperature induced lysogens and plated on Mμ-1 lysogenic WR4027, pin point plaques were found at a frequency of about 10^{-10}. We consider these pin point plaque formers are MuimmP22 hybrids.

Accession For	
NTIS GRA&I	X
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

Contents

Genetics of Novel Hybrid Bacteriophage and Development of Generalized Transducing System for Salmonella typhosa

Annual Progress Report

Nobuto Yamamoto, Ph.D.

February, 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,

FORT DETRICK, FREDERICK, MARYLAND 21701

Hahnemann Medical College
Philadelphia, Pennsylvania 19102

SUMMARY

Length of λimmP22 hybrid genome varies from strain to strain. The genome of λimmP22dis is 5% larger than that of λimmP22 and is tightly packed in the λ protein coat. Therefore λimmP22dis particles are heat unstable. Survivors after heat treatment were often found to be deletion mutants lacking tha al and 9 genes of P22. By employing an approach similar to that previously used to isolate λimmP22 hybrids, we have been able to isolate hybrids between P22 and coliphage ϕ80. We showed the origin of genetic segments in the hybrid phage genomes and suggested that the hybrids are formed as a consequence of multiple crossovers. Moreover, we have been trying to isolate hybrids between P22 and coli mutator phage Mu-1

FOREWORD

Fundamental studies of bacterial and viral genetics not only play an important role in increasing our knowledge of the action of viruses in disease processes, but have contributed greatly to our knowledge of the whole problem of cell replication, genetic transfer, gene control, morphogenesis, and antigen conversion. The significance of the study of bacterial hybrids between E. coli and Salmonella has greatly broadened with the recent discoveries of hybrid phage between coliphage and Salmonella phage. The study supported by this contract will bring many important answers for mechanisms of genetic evolution, transduction, recombination, gene expression, antigen conversion, morphogenesis and viral replication. In addition, such newly constructed hybrids may prove useful in achieving intergeneric transduction via a hybrid phage vector, of chromosomal genes from different genera of interobacteriace. Therefore, such hybrid phages may serve as useful vectors in the genetic engineering of a polyvalent oral attenuated vaccine which expresses immunogenic determinants for antigens of Shigella, Salmonella and perhaps even cholera.

Progress

Present Status of the Project

We have previously reported the isolation of an unusal Salmonella typhimurium hybrid sensitive to coliphage λ and Salmonella phage P22 (Gemski, Baron and Yamamoto, PNAS 69, 3110, 1972). This hybrid, constructed by mating an Escherichia coli-K12 Hfr donor with an S. typhimurium recipient, was characterized as an excellent host for achieving genetic recombination between λ and P22. Two broad hybrid phage classes each with representative types differing presumably in the extent of gene exchange, have been isolated and described in our previous reports. The λimmP22 hybrid class, which has the protein coat of λ, was found to contain at least the c region of P22. The other class, termed P22immλ, has the protein coat of Phage P22 and has inherited at least the c marker of λ. Length of P22 genetic segment in these hybrids varies from strain to strain.

By employing an approach similar to that previously used to isolate λimmP22 hybrids, we have been able to isolate hybrids between P22 and coliphage φ80. These newly isolated hybrid phages φ80immP22 were found to be extremely valuable phages for understanding formation mechanism of hybrids between unrelated phages. Success of isolation of φ80immP22 provides an approach to isolate hybrids between P22 and coli phage Mu-1.

A. Genomic Structure of λimm P22 Hybrids

1. Variation in Genome Size of λimmP22 Hybrids.

A strain of λimmP22dis hybrid was rapidly inactivated in nutrient broth at 55 C whereas λimmP22 was rather stable. Density gradient centrifugation with CsCl revealed that the genome of the λimmP22dis strain contain about 5% larger than that of the λimmP22 strain. These observations suggest that the genome of λimmP22dis hybrid is tightly packed in the λ protein coat, resulting in heat unstability of λimmP22dis particles.

2. Deletion Mutations of λimmP22dis Hybrid

Hybrid λimmP22dis strains carry the gene 9 and al of P22. Their deletion mutants lacking these genes were isolated. Although λimmP22dis hybrid is heat unstable (at 55 C) the deletion mutants are quite heat stable. After heat treatment of λimmP22dis hybrids, survivors were often found to be deletion mutants.

B. Studies of Hybrid Phages between E. coli Phage ϕ80 and Salmonella Phage P22.

1. Isolation and Characterization of Hybrid Phages between E. coli Phage ϕ80 and Salmonella Phage P22.

E. coli-S. typhimurium hybrid stain WR4027 is a rough bacterium and sensitive to coliphage ϕ80 for its replication but insensitive to P22 phage because of lack of P22 phage adsorption. Therefore WR4027 lysogenic for phage ϕ80, WR4027(ϕ80), is insensitive to P22 phage. By infecting WR4027(ϕ80) with a mixture of high titer stocks of rough specific Salmonella phage (designated R phages), we were able to isolate R-phage resistant derivatives of WR4027(ϕ80), designated WR4027(ϕ80)/R, which are smooth and fully sensitive to P22 phage. Phage P22 stocks grown on this smooth derivative of the ϕ80 lysogen give rise to recombinants between P22 and ϕ80. Such recombinants were recovered by plating on a P22 resistant host and immune to ϕ80, namely WR4027(ϕ80). They retain the protein coat of ϕ80 but have acquired the immC region of P22. In addition these ϕ80immP22 recombinant carries P22 DNA replication genes 12 and 18 as well as the x and erf genes of P22. Some ϕ80immP22 recombinants, designate ϕ80immP22dis, contain the immI region as well as the immC region, the two widely separated loci involved in the bipartite immunity system of P22.

2. Characterization of Unusual Hybrid Phages between E. coli phage ϕ80 and Salmonella phage P22.

Although the O-1 antigen conversion gene al and tail gene 9 of P22 are located between immC and immI genes, no ϕ80immP22dis hybrids carry both the al and 9 genes. Some hybrids carry the gene al and others carry the gene 9. (Fig. 1). As shown in Fig.2, both λ and ϕ80 phage genomes contain physically corresponding and functionary similar genes. These phage genomes also carry genetically inert DNA segments which are located

between their respective att and tail (J) genes. However, the entire physical length of the φ80 phage genome is about 92% of the size of λ phage genome. This seems to be reflection of difference in sizes of their inert segments: φ80 carries an inert DNA segment smaller than that of λ (Fig. 2). since the inert segments can be replaced by genes 9 and a1 to form dis hybrid phages, we concluded that the φ80immP22dis hybrid phages are unable to accomodate both genes 9 and a1 simutaneously.

C. Attempts to Isolate Hybrid Phages between P22 and Mutator Phage Mu-1

E. coli - S. typhimurium hybrid strain WR4028 is also insensitive to coliphage Mu-1 for its replication. Therefore we isolated Mu-1 lysogenic strains by infecting WR4027 with phage Mu-1. P22 sensitive derivatives of the above lysogen were also isolated by infecting Mu-1 lysogenic WR4027 with a mixture of high titer stocks of rough specific Salmonella phages (designated R). To isolate MuimmP22 (abbr. Mu-P22) hybrid phage, high titer stocks of P22 grown on WR4027(Mu-1)/R should be plated on WR4027(Mu-1). Numerous attempts to isolate such hybrids were unsuccessful. This may be due to lack of induction of Mu-1 prophage by P22 superinfection because we found that P22 infection of λ or φ80 lysogens results in induction of their prophages. Accordingly we prepared lysogens with temperature inducible (cts) mutants of Mu-1 phage. When P22 phage stocks were prepared by P22 infection of temperature (39^{o}) induced lysogens and plated on Mu-1 lysogenic WR4027, pin point plaques were found at a frequency of about 10^{-10}. We consider these pin point plaque formers are Mu-P22 hybrids.

Publications

Yamamoto, N. Wohlhieter, J.A., Gemski, P. and Baron, L.S. λimmP22dis: A hybrid coliphage λ with both immunity region of Salmonella phage P22, Molecular General Genetics, 166, 233-243, 1978.

Yamamoto, K., Numa, S. Whlhieter, J.A., Gemski, P. and Baron, L.S. Isolation of hybrids between Salmonella phage P22 and coliphage φ80. Abt. Am. Soc. Microbiol. p. 247, 1979.

Yamamoto, N., Gemski, P. and Baron, L.S. 1980. Hybrid phages between Salmonella phage P22 and coliphages: Expression of Salmonella somatic O-1 antigen conversion gene a1 of hybrids phages in E. coli and Shigella. Manuscript in preparation to be submitted to J. Gen. Virol.

Yamamoto, N., Gemski, P. and Baron, L.S. 1980. Variation in genome lengths of λimmP22 hybrids. Manscript in preparation.

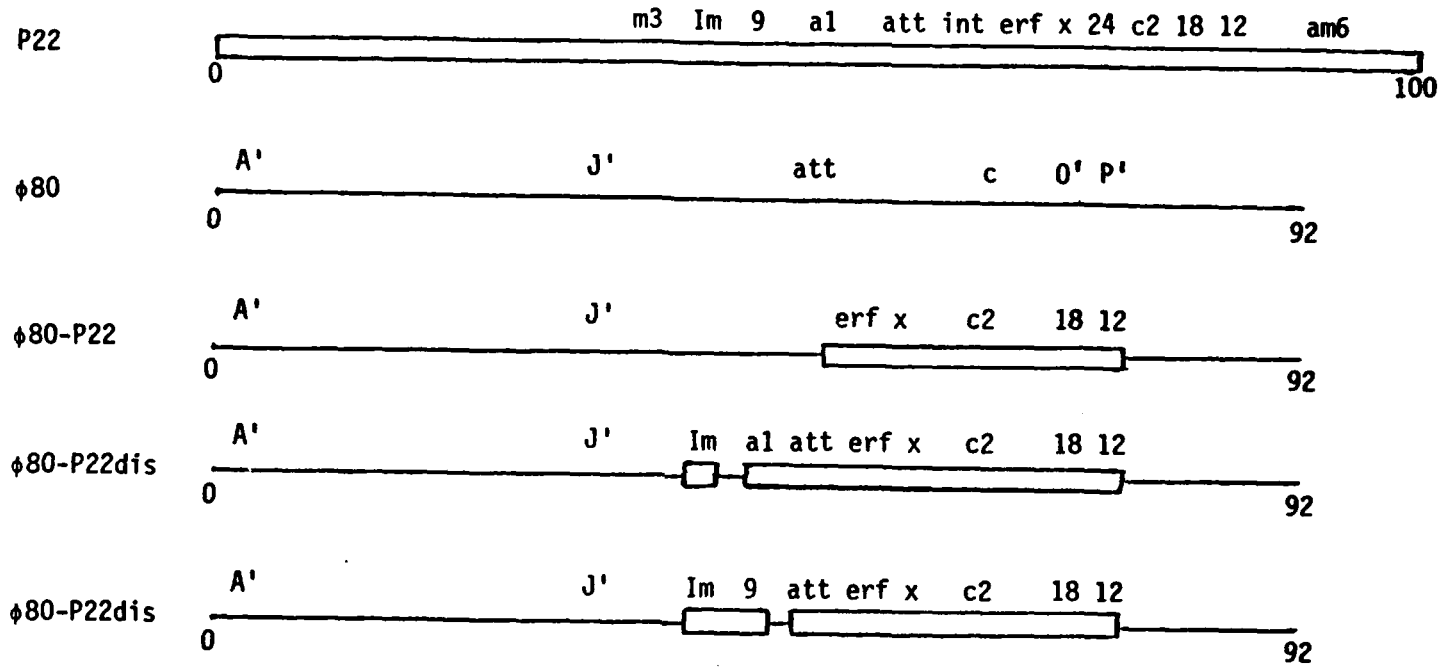


Figure 1. Genome structures of hybrids between Salmonella phage P22 and coliphage φ80.

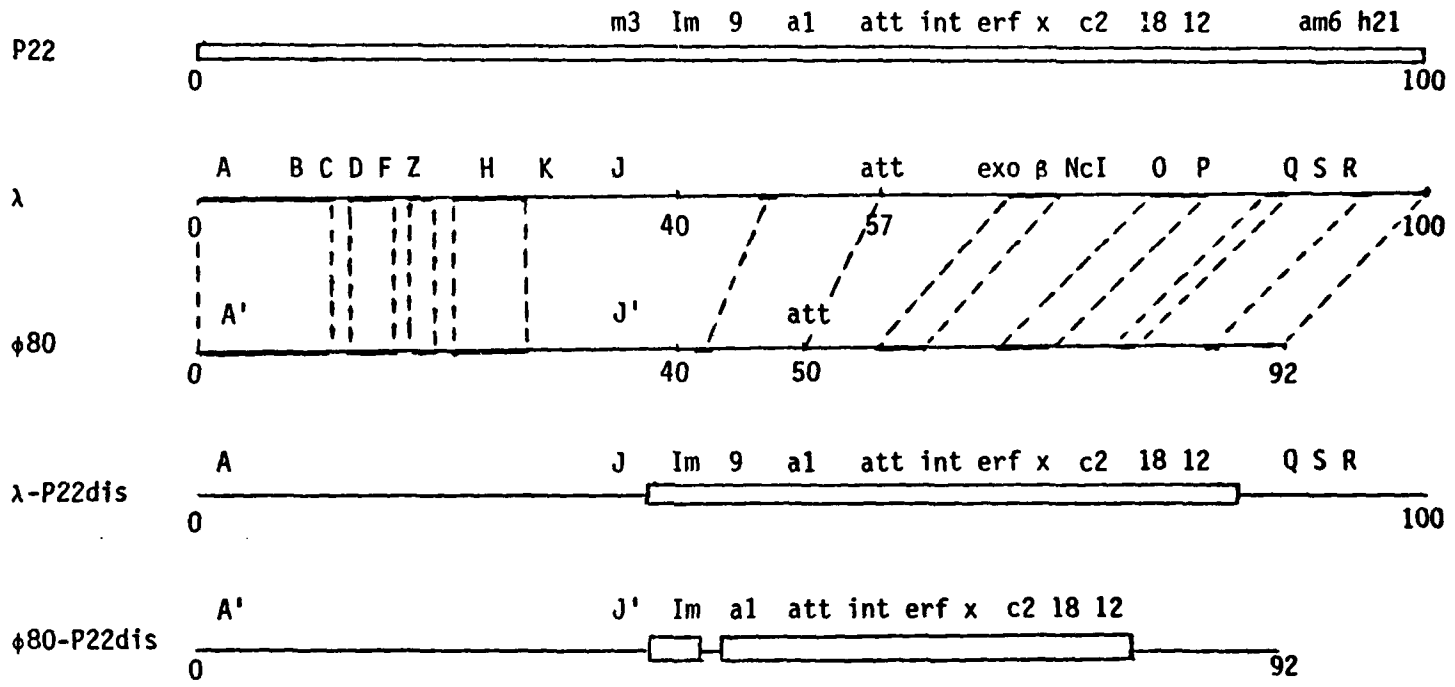


Figure 2. Genome structures of P22, λ, φ80, λimmP22dis and φ80immP22dis phages.

12 Copies	Director (ATTN: SGRD-UWZ-AG) Walter Reed Army Institute of Research Walter Reed Army Medical Center Washington, D.C. 20012
4 Copies	HQDA (SGRD-AJ) Fort Detrick Frederick, Maryland 21701
12 Copies	Defense Documentation Center ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 Copy	Dean School of Medicine Uniformed Services University of Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20014
1 Copy	Superintendent Academy of Health Sciences, U.S. Army ATTN: AHS-COM Fort Sam Houston, Texas 78234

END

DATE
FILMED

5-84

DTIC